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Clinical Correlates in Acromegalic Patients with Pituitary Tumors Expressing GSP Oncogenes

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Abstract. **We herein review published findings on the clinical characteristics of acromegalic patients harboring pituitary somatotrophinomas expressing adenylyl cyclase activating *gsp* mutations and present an update of our own data on a large series of 176 patients with and without these oncogenes. *Gsp* oncogenes are the result of point mutations in either codon 201 or 227 of the G_s-alpha subunit of the G_s-protein which controls adenylyl cyclase. They result ultimately in increased intracellular cAMP levels and thus in excessive growth hormone (GH) secretion. Our large series has allowed us to characterise patients with mutations in codon 201 and the far rarer group possessing codon 227 defects. Both groups were compared with patients without *gsp* oncogenes. In accordance with previous findings, there was no statistically significant difference in age of the patients belonging to each group, the overall average tumor diameter nor in pre-operative serum GH levels, although the latter showed a tendency to be lower in patients with *gsp* oncogenes. The distribution of different types of response during an oral glucose tolerance test (no change, paradoxical rise or greater than 50% decrease in serum GH levels) did not differ between the 3 groups. However, the incidence of microadenomas was higher in acromegalics expressing *gsp* oncogenes in patients possessing mutations in codon 227. Additionally, the incidence of invasiveness was much lower (10% v. 33%) in those tumors with mutations in codon 227. Finally, previous *in-vitro* data indicating that *gsp* oncogene-expressing tumors may respond more efficiently to the somatostatin analogue, octreotide, have been confirmed by subsequent *in-vivo* studies showing a better reduction in serum GH levels in patients with *gsp* oncogenes. These latter findings suggest that presence of *gsp* oncogenes may be a marker for good reponsiveness to octreotide. Assessment of *gsp* oncogene status of surgically removed pituitary somatotrophinomas may thus be helpful in designing optimal medical therapies in those acromegalics requiring further post-operative management of the disease.**

Keywords. **GH, pituitary adenomas, somatotrophinomas, G_s-protein, gsp oncogenes, molecular biology**

Introduction

It is well established that acromegalic patients show a wide spectrum of clinical features. For example, pre-operative serum growth hormone (GH) levels, tumor diameter, response to medical and surgical therapies and post-operative prognosis can all vary considerably between subjects [1,2]. It would be of considerable benefit for clinicians to have available specific markers which allow prediction of these features and thus help in the design of optimal treatment regimens. Especially important would be the ability to accurately decide on the best post-operative management of the disease and to be able to obtain clues on the most likely post-operative course. For several other diseases, specific genetic markers have been successfully used in attaining similar aims [3]. Recent advances in pituitary tumor molecular pathology have suggested that this may eventually also be possible, at least to some degree, for acromegaly [4,5]. Most significantly, clonal analysis has shown that human pituitary somatotrophinomas are the result of defects in specific genes rather than because of abnormalities in external influences [4]. Although intensive efforts are now being made to identify these gene defects, to date only one type, *gsp* oncogenes, has been fully characterised [5,6].

Gsp oncogenes are the result of point mutations and they occur in about 40% of somatotrophinomas. It is almost certain that the remaining 60% of somatotrophinomas will prove to be a heterogenous group, with a variety of genetic defects accounting for tumorigenesis. It might be anticipated, therefore, that it will be possible to sub-group somatotrophinomas in terms of specific molecular defects and it would not be surprising if each specific defect could be correlated to clinical and prognostic parameters. At present, it is possible to compare only one homogenous group of somatotrophinomas, those with *gsp* oncogenes, with the remaining heterogenous group of somatotrophinomas. Although the conclusions which can be drawn from such comparisons is limited, it is nevertheless becoming clear that some features of somatotrophinomas expressing *gsp* oncogenes may be of clinical relevance in terms of prognosis and deciding optimal post-operative treatment [2,7–11]. We shall review these developments and

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present an update of results of our latest series comprising 176 acromegalics examined for presence or absence of *gsp* oncogenes.

GSP *Oncogenes*

The discovery of *gsp* oncogenes was based upon the observation that about 30–40% of GH-secreting secreting pituitary somatotrophinomas possess elevated adenylyl cyclase activity, and hence increased intracellular cAMP levels [12]. Biochemical analyses indicated that this is due to a defect in the alpha-subunit of the G_s protein (G_s-alpha) which controls adenylyl cyclase activity. In a logical progression, sequence analysis of the G_s-alpha gene in somatotrophinomas with increased intracellular cAMP levels revealed point mutations in regions coding for critical amino-acids in the mature polypeptide [5,6]. These mutations occur in either codon 201 or, more rarely, codon 227. In the former, the wild type CGT, encoding arginine, can mutate to either TGT (cysteine) or CAT (histidine). The wild type codon 227, CAG (glutamine) can mutate to CGG or CTG, encoding for arginine or leucine, respectively. All these amino-acid substitutions lead to a disrupted G_s protein structure such that adenylyl cyclase activity becomes permanently active.

Both types of mutation ultimately result in increased intracellular cAMP levels by inhibiting the intrinsic GTPase activity of the G_s-alpha subunit [5,13]. The normal arginine at position 201 is the residue which is ADP-ribosylated by cholera toxin, a covalent modification which also inhibits GTPase activity [5]. The normal glutamine residue at position 227 is, however, in a guanine nucleotide binding region [5]. Thus, the precise molecular mechanisms by which the amino-acid substitutions abolish the intrinsic GTPase activity of G_s-alpha presumably differ, and it may be of significance that the 201 mutations are far more common than those occurring in 227 [2,5–7,10,11]. Most studies indicate that mutations in codons 201 and 227 occur with a frequency of 30% and 5–10%, respectively. Because of the rarity of somatotrophinomas with mutations in codon 227, it has not yet been possible to compare accurately the characteristics of these types of tumor with those containing mutations in codon 201. Our present large series has provided the opportunity to compare these two types of tumor.

GSP *Oncogenes and Clinical Correlates*

To gain insight into the detailed biology and potential clinical significance of *gsp* oncogenes, attempts have been made to determine and compare the characteristics of acromegalics with and without these mutations [2,7–11,14]. To date, results concerning tumor size and pre-operative serum GH levels have been conflicting. Somatotrophinomas with *gsp* oncogenes were reported to be smaller in 2 studies [10,11] but this was not confirmed by two other groups [2,14]. Likewise, basal serum GH levels in acromegalics with *gsp* oncogenes have been conflictingly reported to be higher [10], lower [11] or not significantly different [2] than those found in patients without the mutations. To date, there have been no comparisons on tumor invasiveness and recurrence. Additionally, no studies have attempted to separate and compare acromegalics with mutations in codons 201 and 227. Our present series has allowed us to perform such comparisons. Genomic DNA was extracted from the tumors and presence of *gsp* mutations determined by sequence analysis of the G_s-alpha gene following its amplification by the polymerase chain reaction (PCR), as previously described in detail [2,7]. The finding of a double band in the appropriate codon confirmed presence of a mutation (Fig. 1). Of the 176 patients, 71 (40.3%) possessed *gsp* oncogenes. The breakdown of the 4 different types of point mutation in the 71 somatotrophinomas is summarised in Fig. 2. Mutations in codon 201 were far more frequent (34%) with the transition CGT TGT being the most common alteration. Specifically, 201TGT, 201CAT, 227CGG and 227CTG type mutations were found in 47, 13, 2 and 9 tumors, respectively. Thus, only 11 (6.3%) tumors possessed mutations in codon 227.

The average age of acromegalics without *gsp* oncogenes and those with mutations in codons 201 and 227 did not significantly differ (without *gsp* oncogenes:43.2 ± 13.6; with mutations in codons 201 and 227: 48.9 ± 12.5 and 51.4 ± 14.8, respectively, results expressed years as mean ± SD). Although average preoperative serum GH levels tended to be lower in the groups expressing *gsp* oncogenes (without *gsp* oncogenes: 62.8 ± 84.8; with mutations in codons 201 and 227: 41.1 ±

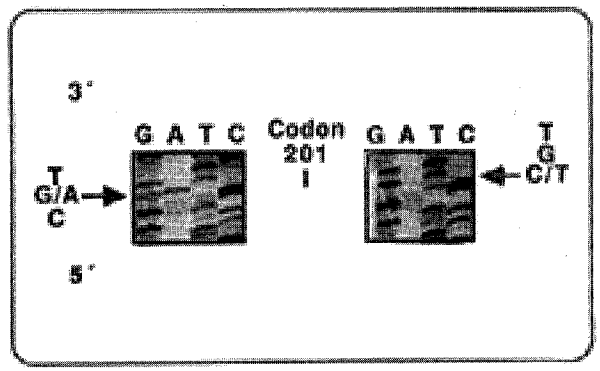

Fig. 1. *Sequence of codon 201 of the G_s-alpha gene obtained by PCR of genomic DNA extracted from 2 human pituitary somatotrophinomas expressing gsp oncogenes (left and right boxes). In both cases, the wild-type allele reads CGT (arginine). The double bands (arrowed) represent the wild type and mutant forms in each case. The left box shows a 201CAT (arginine histidine) type mutation, and the right box shows a 201TGT (arginine cysteine) type mutation.*

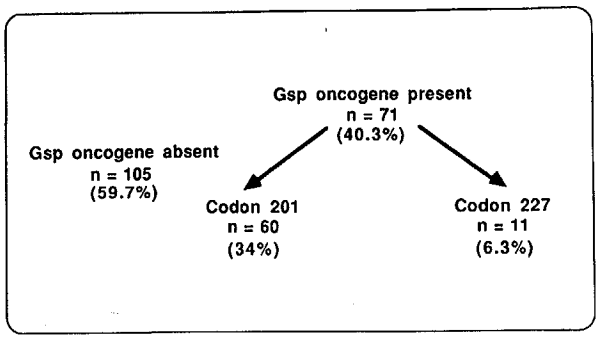

Fig. 2. *Breakdown of 176 acromegalic patients into those without gsp oncogenes and those with mutations in codons 201 and 227 of the G_s-alpha gene.*

46.1 and 28.3 ± 27.2, respectively, results expressed as mean ± SD ng/ml), these differences were not statistically significant because of the very high degree of variation between individual patients. However, on examination of the data available from 164 of the patients, there appeared to be a higher incidence of microadenomas within the *gsp* oncogene-expressing groups (Fig. 3). Thus, only 7.2% of tumors without the mutations were microadenomas, whereas the incidence was 19% and 30% in the groups with mutations in codons 201 and 227, respectively. These higher rates were significantly ($P < 0.05$ by Chi-square analysis) different compared with the *gsp* negative group. Additionally, the incidence of invasiveness was much lower in tumors with mutations in codon 227, being only 10% as compared with 33% in tumors without *gsp* oncogenes or with mutations in codon 201 (Fig. 4). This finding may be related to the considerably higher occurrence rate of microadenomas in this group. It might also be of relevance that none of the tumors with mutations in codon 227 were treated for recurrent or persistent

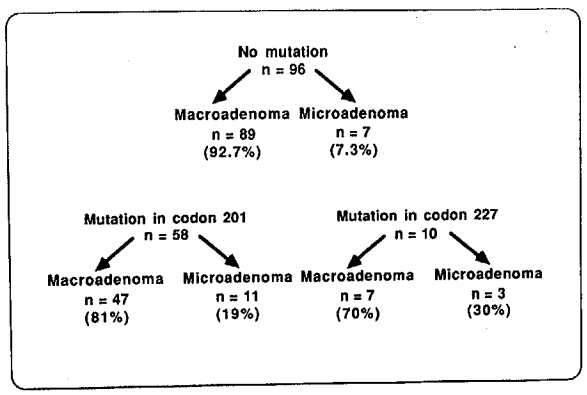

Fig. 3. *Breakdown of 164 (somewhat accurred with the number 174) acromegalic patients into those with macroadenomas and microadenomas in relation to presence or absence of mutations in codons 201 and 227 of the G_s-alpha gene.*

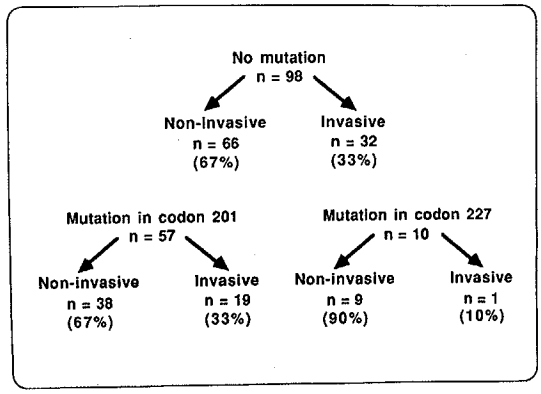

Fig. 4. *Breakdown of 165 acromegalic patients into those with non-invasive and invasive pituitary somatotrophinomas in relation to presence or absence of mutations in codons 201 and 227 of the G_s-alpha gene.*

acromegaly whereas the overall rate of tumour tissue obtained during reoperation was 15% in this study. Despite these differences in incidence of microadenomas, invasiveness and tumor diameters as measured from the pre-treatment MR-Scans did not significantly differ between the 3 groups (without *gsp* oncogenes: 19.2 ± 8.4; with mutations in codons 201 and 227: 19.1 ± 8.9 and 12.2 ± 3.4, respectively, results expressed as mean ± SD in mm).

Pre-Operative Serum GH Levels during an Oral Glucose Tolerance Test

Previous studies have suggested that acromegalics with somatotrophinomas expressing *gsp* oncogenes do not exhibit a paradoxical rise in serum GH levels during an oral glucose tolerance test (oGTT) [2,10]. Additionally, the suppressive effect on GH secretion tended to be greater in the *gsp* oncogene expressing group. However, these conclusions were based on data obtained from relatively small groups of patients. Serum GH levels during an oGTT were available on 145 of the acromegalics in the present series. Eighty-nine of these did not possess *gsp* oncogenes, whereas 47 and 9 patients possessed mutations in codons 201 and 227, respectively. In the group without mutations, serum GH levels were either not altered, paradoxically increased or showed a greater that 50% decrease in 52 (58%), 21 (23%) and 16 (18%) of cases, respectively. An almost identical distribution of response were observed in the patient group expressing *gsp* oncogenes. Of the 56 patients, 31 (55%) showed no response during the oGTT, 17 (30%) exhibited a paradoxical rise and 8 (14%) showed a decrease in serum GH levels. There was no difference in this distribution between patients with mutations in codons 201 and 227. Thus, the data

from this large series of patients appears to contradict the earlier suggestions of more efficient response to an oGTT of acromegalics with *gsp* oncogenes.

Effect of Octreotide in Acromegalics With and Without GSP Oncogenes

Octreotide is a long acting analogue of somatostatin and is able to decrease serum GH levels in most acromegalics [17]. However, the magnitude of response to octreotide varies considerably between patients and in some cases it fails to significantly inhibit GH secretion. Evidence is accumulating that a large proportion of those acromegalics in whom octreotide exerts a powerful inhibitory effect are those harboring *gsp* oncogenes [7,8,9,16]. In an initial *in-vitro* study, treatment of cultured human pituitary somatotrophic tumor cells with octreotide resulted in consistent and strong inhibition of GH secretion by all tumors possessing *gsp* oncogenes [7]. In contrast, over 50% of tumors without *gsp* oncogenes failed to respond to octreotide. Because of these findings, it was suggested that presence of *gsp* mutations may be equated with a marker for good responsiveness to octreotide in acromegaly [7,13]. Further support for this concept was provided by 2 *in-vivo* studies, in which it was shown that that pre-operative administration of octreotide to acromegalics reduced serum GH levels by a far greater amount in those patients subsequently found to possess *gsp* oncogenes than those without the mutations [8,9]. The earlier *in-vitro* findings were also confirmed by one of these studies [9].

These findings with octreotide are consistent with the concept that inhibition of GH secretion by somatostatin and its analogues is mediated via activation of G_i, the G-protein which is able to reduce adenylyl cyclase activity [17]. Indeed, somatostatin results in more efficient reduction of adenylyl cyclase activity in *gsp* oncongene-expressing tumors than those without the mutations [12]. The reasons for non-responsiveness to octreotide in some of the latter tumors remains unknown but may be related to decreased somatostatin receptor concentration or a change in the distribution of receptor sub-types [16].

Summary

Based on data obtained from a large series of human pituitary somatotrophinomas surgically resected from patients with acromegaly and gigantism, we have been able to better characterise the clinical correlates of tumors expressing *gsp* oncogenes. The most striking difference between tumors with and without *gsp* mutations is that the former exhibit a consistent and increased sensitivity to octreotide. This may have clinical significance in terms of predicting optimal post-operative management of acromegaly. Additionally, we have provided some evidence that the incidence of microadenomas is greater in the group of tumors possessing *gsp* oncogenes, and that invasiveness occurs very rarely in those with mutations in codon 227. However, there appears to be no obvious difference between the groups of tumor in terms GH secretory activity and average age of patients.

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